(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2008 (03.01.2008)

PCT

(10) International Publication Number
WO 2008/002480 A2

(51) International Patent Classification:
A01H 5/00 (2006.01) *C12N 5/04* (2006.01)
C07H 21/04 (2006.01)

(21) International Application Number:
PCT/US2007/014580

(22) International Filing Date: 23 June 2007 (23.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/816,086 23 June 2006 (23.06.2006) US

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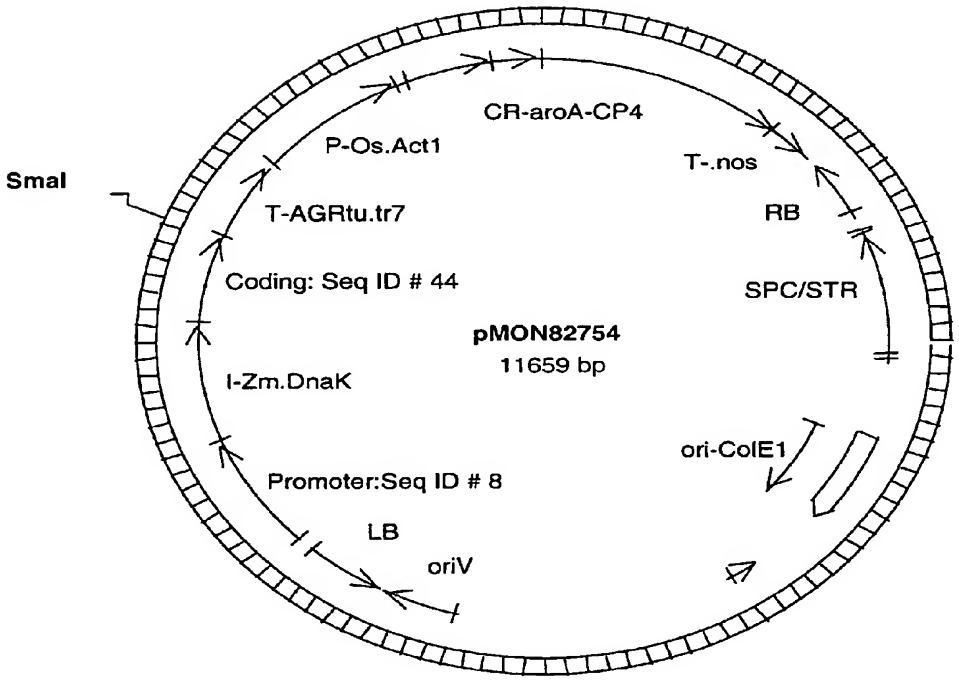
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: TRANSGENIC CROP PLANTS WITH IMPROVED STRESS TOLERANCE



(57) Abstract: Disclosed herein are novel compositions of NF-YB proteins and recombinant DNA for expressing NF-YB proteins that are used to produce transgenic plants with enhanced yield and/or enhanced water deficit stress tolerance.

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— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

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TRANSGENIC CROP PLANTS WITH IMPROVED STRESS TOLERANCE

CROSS –REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority under 35USC § 119(e) of United States Provisional Application Serial No. 60/816,086 filed June 23, 2006, which is herein incorporated by reference in its entirety.

INCORPORATION OF SEQUENCE LISTINGS

[0002] A Computer Readable Form of the Sequence Listing (on CD-ROM containing the file named "54485B.ST25.txt" which is 70 KB as measured in MS-WINDOWS operating system and was created on June 22, 2007) is incorporated herein by reference.

FIELD OF THE INVENTION

[0003] Disclosed herein are transgenic plant cells in seeds and plants with improved stress tolerance and methods of making and using such cells, seeds and plants.

BACKGROUND OF THE INVENTION

[0004] Crop plant yield is reduced by any of a number of biotic or abiotic stresses on such plants. Growers have used a variety of strategies to minimize adverse effects from stress. For instance, stress from weeds can be reduced by application of herbicides, stress from insects can be reduced by application of pesticides, stress from water deficit can be reduced by irrigation, stress from cold can be reduced by delaying planting time, and stress from nutrient deficiency can be reduced by treating with fertilizer.

[0005] The yield from a plant is influenced by environmental factors including water availability, exposure to cold or heat, availability of nutrients such as phosphorus and nitrogen, plant density, and the like. A plant's response to such environmental stress can be influenced by internal genetic mechanisms. An object of plant genetic

engineering is to produce novel plants with agronomically, horticulturally, or economically important traits including increased tolerance to any of a variety of environmental stresses.

[0006] Considering the complexity of water use in land plants, especially during conditions that produce water deficit, relatively few genes specifically associated with this aspect of physiology have been identified. The use of recombinant DNA expressing certain Hap3 CAAT box DNA binding transcription factors for improving water deficit tolerance is disclosed in US 2005/0022266 A1. Hap3 transcription factors are also known as NF-YB proteins which form complexes with NF-YA and NF-YC proteins in plants. These proteins are collectively referred to as NFY proteins. The amino acid sequence of a corn NF-YB protein is SEQ ID NO:28.

SUMMARY OF THE INVENTION

[0007] This invention provides novel plant chromosomal DNA comprising a recombinant polynucleotide that provides for expression of an NF-Y protein that provides protection against water deficit stress conditions. Such plant chromosomal DNA is flanked by native plant DNA. To accommodate the vagaries of nature, embodiments of the plant chromosomal DNA can provide plants with improved yield as compared to control crop plants when the plants are grown in water deficit stress conditions, as well as comparable or improved yield as compared to control crop plants when grown in water sufficient conditions. This invention also provides transgenic plant cells, plants, seeds and crops having the novel plant chromosomal DNA of this invention.

[0008] A characteristic of the plant chromosomal DNA segments used in this invention is the presence of a recombinant polynucleotide that encodes for low expression, e.g. constitutive expression at a level close to background expression of a native NF-Y protein, i.e. where NF-Y protein is produced in leaf cells at a level up to 40 picograms per microgram of total protein in plant leaf tissue cells or less, e.g. up to about 30 or 20 picograms per microgram of total protein in plant leaf tissue cells. In other embodiments the NF-Y protein expressed from the recombinant polynucleotide is in the range of 0.1 to 11 picograms per microgram of total protein in plant leaf tissue cells.

[0009] In some embodiments of the invention the recombinant polynucleotide provides for expression of at least one NF-Y protein and a marker protein. In another embodiment the recombinant polynucleotide provides for expression of a single NF-Y protein.

[0010] In some embodiments the NF-Y protein expressed by the recombinant polynucleotide is a native NF-YA, NF-YB or NF-YC protein. In other embodiments the NF-Y protein expressed by the recombinant polynucleotide is an exogenous NF-YA, NF-YB or NF-YC protein, e.g. an NF-YB protein from *Arabidopsis thaliana.* In yet, other embodiments the NF-Y protein expressed by the recombinant polynucleotide is a variant of a native NF-YA, NF-YB or NF-YC, e.g. in corn plant chromosomal DNA the recombinant polynucleotide expresses an variant NF-YB protein comprising contiguous amino acids from native corn DNA sequence.

[0011] The plant chromosomal DNA segments of this invention are provided by employing any number of low level constitutive promoters for expression of the NF-Y protein. Such promoters are readily identified and isolated by those skilled in the art and can include a promoter selected from the group consisting of a rice alpha tubulin promoter, a rice actin promoter, a PPDK mesophyll tissue-enhanced promoter, and a rubisco activase bundle sheath tissue-enhanced promoter.

[0012] Such plant chromosomal DNA of this invention is useful in transgenic plant cells of plants that are desired to exhibit water deficit stress tolerance. Such plant chromosomal DNA is useful in providing a transgenic crop of water deficit stress tolerant plants. e.g. where the harvested yield of said crop is enhanced over the yield of a crop of control plants not having said plant chromosomal DNA segment. Because of the unpredictability of rainfall and/or availability of irrigation water, plants of this invention may be grown in a wide range of water sufficiency conditions, ranging from water sufficient conditions over the lifetime of the plant to various levels of water deficit stress conditions over the lifetime of the plant. To accommodate such variation in water sufficiency, certain embodiments of this invention provide transgenic crops that exhibit increased harvested yield as compared to control crop plants when the plants are grown in water deficit stress conditions, as well as comparable or increased yield as compared to control crop plants when grown in water sufficient conditions. Such crops in include

water deficit-tolerant plants of corn, cotton, soybean, sugarcane, switchgrass, rice, wheat, alfalfa, or canola plants. In one aspect of the invention the plant chromosomal DNA is in a transgenic corn plant and comprises recombinant polynucleotides for expressing an NF-YB protein which is a native corn protein or a variant thereof.

[0013] Another aspect of this invention provides transgenic pollen grains comprising a haploid derivative of a plant cell containing a plant chromosomal DNA segment of this invention. Another aspect of this invention is anti-counterfeit milled seed having, as an indication of origin, a plant cell with said chromosomal DNA segment of this invention.

[0014] Still other aspects of this invention provide methods of improving water deficit stress tolerance and yield in a crop plant line comprising providing in the genome of a crop plant line a plant chromosomal DNA segment of this invention.

[0015] Another method of this invention provides for the manufacture of non-natural, transgenic seed or propagules that can be used to produce a crop of transgenic plants with enhanced water deficit tolerance resulting from expression of an NF-YB protein from a plant chromosomal DNA segment of this invention. Such a method comprises screening a population of plants having such plant chromosomal DNA segment and control plants for said enhanced yield when grown under water-deficit stressed and enhanced or comparable yield when grown under water sufficient conditions, selecting from said population one or more plants that exhibit enhanced yield as compared to the yield for control plants under water-deficit stressed or enhanced or comparable yield as compared to the yield for control plants when grown under water sufficient conditions, verifying that said plant chromosomal DNA segment is stably integrated in said selected plants, analyzing leaf tissue of a selected plant to determine the production of transgenic NF-YB protein at a level up to 40 picograms of NF-YB protein per microgram of total protein in said leaf tissue, and collecting seed or a regenerative propagule from a selected plant.

[0016] Another method provides for the production of inbred corn seed comprising acquiring hybrid corn seed from a herbicide tolerant corn plant which also has stably-integrated, chromosomal DNA segment of this invention, introgressing the chromosomal DNA segment from said acquired hybrid corn seed into a second corn line

by allowing pollen grains comprising a haploid derivative with said chromosomal DNA segment to pollinate said second corn line to produce crossed seeds, producing a population of plants from crossed seeds (where a fraction of the seeds produced from said pollination is homozygous for the chromosomal DNA segment, a fraction is hemizygous, and a fraction does not have the chromosomal DNA segment), selecting corn plants which are homozygous and hemizygous for said chromosomal DNA segment by treating with an herbicide, collecting seed from herbicide-treated-surviving corn plants and planting said seed to produce further progeny corn plants, and backcrossing plants grown from said progeny seeds with said second corn line to produce an inbred corn line. The method can be further employed by crossing the inbred corn line with a third corn line to produce hybrid seed.

[0017] Yet another aspect of this invention provides a method of growing a corn, cotton, soybean, sugarcane, switchgrass, rice, wheat, alfalfa, or canola crop without irrigation water comprising planting seed having plant cells with a plant chromosomal DNA segment of this invention, where the seeds are produced from plants that are selected for enhanced water deficit stress tolerance.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 is a plasmid map for plant transformation vector pMON82754.

[0019] Figure 2 is a plasmid map for plant transformation vector pMON63796.

[0020] Figure 3 illustrates the yield performance of plants under water deficit stress conditions where different promoters are used in recombinant polynucleotides for expressing an NF-Y protein.

[0021] Figure 4 illustrates the yield performance of plants under water sufficient conditions where different promoters are used in recombinant polynucleotides for expressing an NF-Y protein.

DETAILED DESCRIPTION OF THE INVENTION

[0022] Unless specifically defined, all technical and scientific terms used herein have the same meaning as commonly understood by persons of ordinary skill in the art.

The procedures for preparing and screening transgenic plants described below are well known and commonly employed by persons of ordinary skill in the art.

[0023] As used herein "water deficit" means a period when water available to a plant is not replenished at the rate at which it is consumed by the plant. A long period of water deficit is colloquially called drought. Lack of rain or irrigation may not produce immediate water stress if there is an available reservoir of ground water for the growth rate of plants. Plants grown in soil with ample groundwater can survive days without rain or irrigation without adverse affects on yield. Plants grown in dry soil are likely to suffer adverse affects with minimal periods of water deficit. Severe water deficit stress can cause wilt and plant death; moderate drought can cause reduced yield, stunted growth or retarded development. Plants can recover from some periods of water deficit stress without significantly affecting yield. However, water deficit stress at the time of pollination can have an irreversible effect in lowering yield. Thus, a useful period in the life cycle of corn for observing water deficit stress tolerance is the late vegetative stage of growth before tasseling. Water deficit stress tolerance is determined by comparison to control plants. For instance, plants of this invention can survive water deficit stress with a higher yield than control plants. In the laboratory and in field trials drought can be simulated by giving plants of this invention and control plants less water than is given to sufficiently-watered control plants and measuring differences in traits.

[0024] A suitable control plant may be a non-transgenic plant of the parental line used to generate a transgenic plant herein. A control plant may in some cases be a transgenic plant line that includes an empty vector or marker gene, but does not contain the recombinant polynucleotide of the present invention that is expressed in the transgenic plant being evaluated. A control plant in other cases is a transgenic plant expressing the gene with a constitutive promoter. In general, a control plant is a plant of the same line or variety as the transgenic plant being tested, lacking the specific trait-conferring, recombinant DNA that characterizes the transgenic plant. Such a progenitor plant that lacks that specific trait-conferring recombinant DNA can be a natural, wild-type plant, an elite, non-transgenic plant, or a transgenic plant without the specific trait-conferring, recombinant DNA that characterizes the transgenic plant. The progenitor plant lacking the specific, trait-conferring recombinant DNA can be a sibling of a

transgenic plant having the specific, trait-conferring recombinant DNA. Such a progenitor sibling plant may include other recombinant DNA.

[0025] As used herein "yield" of a crop plant means the production of a crop, e.g., shelled corn kernels or soybean or cotton fiber, per unit of production area, e.g., in bushels per acre or metric tons per hectare, often reported on a moisture adjusted basis, e.g., corn is typically reported at 15.5 % moisture. Moreover a bushel of corn is defined by law in the State of Iowa as 56 pounds by weight, a useful conversion factor for corn yield is: 100 bushels per acre is equivalent to 6.272 metric tons per hectare. Other measurements for yield are in common practice.

[0026] A transgenic "plant cell" means a plant cell that is transformed with stably-integrated, non-natural, recombinant polynucleotides, e.g. by *Agrobacterium*-mediated transformation or by bombardment using microparticles coated with recombinant polynucleotides. A plant cell of this invention can be an originally-transformed plant cell that exists as a microorganism or as a progeny plant cell that is regenerated into differentiated tissue, e.g. into a transgenic plant with stably-integrated, non-natural recombinant polynucleotides in its chromosomal DNA, or seed or pollen derived from a progeny transgenic plant.

[0027] A "transgenic" plant or seed means one whose genome has been altered by the stable incorporation of recombinant polynucleotides in its chromosomal DNA, e.g. by transformation, by regeneration from a transformed plant from seed or propagule or by breeding with a transformed plant. Thus, transgenic plants include progeny plants of an original plant derived from a transformation process including progeny of breeding transgenic plants with wild type plants or other transgenic plants. The enhancement of a desired trait can be measured by comparing the trait property in a transgenic plant which has recombinant DNA conferring the trait to the trait level in a progenitor plant. Although many varieties of plants can be advantageously transformed with recombinant DNA for expressing an NF-YB protein to provide water stress tolerance and/or enhanced yield, especially useful transgenic plants with water stress tolerance include corn (maize), soybean, cotton, canola (rape), wheat, rice, alfalfa, sorghum, grasses such as switchgrass, vegetables and fruits.

[0028] "Expressing a protein" means the function of a cell to transcribe recombinant DNA to mRNA and translate the mRNA to a protein. For expression the recombinant DNA usually includes regulatory elements including 5' regulatory elements such as promoters, enhancers, and introns; other elements can include polyadenylation sites, transit peptide DNA, markers and other elements commonly used by those skilled in the art. Promoters can be modulated by proteins such as transcription factors and by intron or enhancer elements linked to the promoter. Promoters in recombinant polynucleotides can also be modulated by nearby promoters. For example, the activity of a low constitutive promoter as used in this invention can be significantly increased to undesirably high levels by the activity of a second highly expressing promoter in the recombinant polynucleotide. For instance, there is disclosed in US 2005/0022266 A1 a recombinant polynucleotide construct a transcription unit comprising a low level-expressing rice actin promoter operably linked to DNA coding for an NF-YB protein followed by a transcription unit comprising a CaMV 35S promoter operably linked to DNA coding for the nptII marker. Although the plants having such recombinant polynucleotides in the chromosomal DNA exhibited water deficit stress tolerance, the CaMV 35S promoter is able to enhance the otherwise low expression of the nearby rice actin promoter to such high levels as to cause a reduction in yield in plants grown under water sufficient conditions. An aspect of this invention involves the use of recombinant polynucleotides having promoters for expressing NF-Y proteins at low levels to avoid reduction in yield when plants are grown under water sufficient conditions.

[0029] "Recombinant polynucleotide" means a DNA construct that is made by combination of two otherwise separated segments of DNA, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. Recombinant DNA can include exogenous DNA or simply a manipulated native DNA. Recombinant DNA for expressing a protein in a plant is typically provided as an expression cassette which has a promoter that is active in plant cells operably linked to DNA encoding a protein, e.g. an NF-YB protein, linked to a 3' DNA element for providing a polyadenylation site and signal. Useful recombinant DNA also includes expression cassettes for expressing one or more proteins conferring herbicide tolerance and/or insect resistance. With reference to the sequence listing the DNA of various

promoters are identified in Table 1 and the DNA encoding various embodiments of NF-YB proteins are identified in Table 2. Certain genes encoding native NF-YB subunits are identified using "Gnnnn" nomenclature, e.g. the *Arabidopsis thaliana* G481 gene. A set of useful promoters is disclosed in Table 1 with reference to DNA in the sequence listing for the promoter element including enhancer, leader and intron elements used in various illustrative embodiments. These and numerous other promoters that function in plant cells are known to those skilled in the art and available for use in alternative embodiments of this invention to provide for expression of NF-Y proteins in transgenic plant cells.

Table 1

Promoter Expression (species of origin)	SEQ ID
Ubiquitous- high in leaf but lower in reproductive tissue (CaMV35S-enhanced)	1 and 2
Ubiquitous- high in leaf but lower in reproductive tissue (CaMV35S)	3
Epidermis, stomatal guard cells enhanced (rice)	4
Silk enhanced (corn)	5
Silk enhanced (sorghum)	6
Constitutive - low level (corn)	7
Bundle sheath enhanced (corn RUA)	8
Drought inducible (corn)	9
Mesophyll enhanced (corn)	10
Drought inducible (corn)	11
Root enhanced (pea)	12
Ubiquitous (rice alpha tubulin)	13
Ubiquitous (rice actin1)	14
Ubiquitous (rice actin1)	15
Root enhanced (corn NAS2)	16
Embryo (barley)	17
Ubiquitous (Arabidopsis)	18
Drought inducible (Arabidopsis)	19
Green tissue enhanced (Arabidopsis)	20
Drought inducible (Arabidopsis)	21
Drought inducible (Arabidopsis)	22
Drought inducible (Arabidopsis)	23
Green tissue enhanced (Arabidopsis)	24
Root enhanced (Arabidopsis)	25
Vascular tissue enhanced (Arabidopsis)	26
Chimeric bundle sheath/mesophyll enhanced (corn)	27
Ubiquitous (Arabidopsis EF-1 alpha)	60
Seed (Glycine max)	61

Table 2

Arbitrary DNA Name	SEQ ID	Protein Features
Corn NF-YB2-S83A	29	In SEQ ID NO:28 Serine at position 83 is changed to Alanine
Corn NF-YB2-123C	30	Methionine and amino acids 29-134 of SEQ ID NO:28 representing domains 1, 2, 3 and C of the protein
Corn NF-YB2- 23C	31	Methionine and amino acids 68-134 of SEQ ID NO:28 representing domains 2, 3 and C of the protein
Corn NF-YB2-C73:89S	32	In SEQ ID NO:28 Cysteines at positions 73 and 89 are changed to Serine
Corn NF-YB2-C73R:C89S	33	In SEQ ID NO:28 Cysteine at position 73 changed to Arginine, and Cysteine at position 89 is changed to Serine
Corn NF-YB2-C73S:C89S:L102R	34	In SEQ ID NO:28 Cysteine at position 73 is changed to Serine, Cysteine at position 89 is changed to Serine, and Leucine at 102 is changed to Arginine
Corn NF-YB2-E76R:S83R	35	In SEQ ID NO:28 Glutamate at position 76 is changed to Arginine and Serine at position 83 is changed to Arginine
Corn NF-YB2-I115A	36	In SEQ ID NO:28 Isoleucine at position 115 is changed to Alanine
Corn NF-YB2-I49R:C73R:C89S:L102R	37	In SEQ ID NO:28 Isoleucine at position 49 is changed to Arginine, Cysteine at position 73 is changed to Arginine, Cysteine at position 89 is changed to Serine, and Leucine at position 102 is changed to Arginine
Corn NF-YB2-I49R:C73S:C89S	38	In SEQ ID NO:28 Isoleucine at position 49 is changed to Arginine, Cysteine at position 73 is changed to Serine, and Cysteine at position 89 is changed to Serine
Corn NF-YB2-L102A	39	In SEQ ID NO:28 Leucine at position 102 is changed to Alanine
Corn NF-YB2-L103A	40	In SEQ ID NO:28 Leucine at position 103 is changed to Alanine
Corn NF-YB2-L109A	41	In SEQ ID NO:28 Leucine at 109 is changed to Alanine
Corn NF-YB2-L118A	42	In SEQ ID NO:28 Leucine at 118 is changed to Alanine
Corn NF-YB2-L122A	43	In SEQ ID NO:28 Leucine at 122 is changed to Alanine

Arbitrary DNA Name	SEQ ID	Protein Features
Corn NF-YB2 (PHE0000004)	44	NF-YB protein of SEQ ID NO:28
Corn NF-YB2a (PHE0008666)	45	Amino acids TPIANGK at 55-61 of SEQ ID NO:28 are deleted
Corn.NFB2-1:4:9 (PHE0008660)	46	Corn NF-YB2 protein
soybean G482-like 3 (PHE0001202)	47	Soybean NF-YB protein
Soybean NF-YB (G481 like) (G3472)	48	Soybean NF-YB protein
Gm.G481-1:1:1 (PHE0010412)	49	Soybean NF-YB protein
soy G481-like 3 (PHE0003740)	50	Soybean NF-YB protein
soybean G482-like 1 (PHE00001201)	51	Soybean NF-YB protein
Soy G1820 like (PHE0003227)	52	Soybean NF-YB protein
Arabidopsis G481 (PHE0000002)	53	Arabidopsis NF-YB protein
Arabidopsis G1364 (PHE0003728)	54	Arabidopsis NF-YB protein
Arabidopsis G485 (PHE0010350)	55	Arabidopsis NF-YB protein
GhG481-1:1:1 (PHE0010352)	56	Cotton NF-YB protein
Gh.NFB2-1:1:1 (PHE0010354)	57	Cotton NF-YB protein
CR-Gm.G481-6-1:1:1 (PHE0003701)	58	Soybean NF-YB protein
Os.NFYB2 (PHE0004246)	59	Rice NF-YB protein

[0030] Recombinant DNA constructs generally include a 3' element that typically contains a polyadenylation signal and site. Well-known 3' elements include those from *Agrobacterium tumefaciens* genes such as *nos 3'*, *tml 3'*, *tmr 3'*, *tms 3'*, *ocs 3'*, *tr7 3'*, *e.g.*, disclosed in U.S. 6,090,627. 3' elements from plant genes such as wheat (*Triticum aestivum*) heat shock protein 17 (*Hsp17 3'*), a wheat ubiquitin gene, a wheat fructose-1,6-biphosphatase gene, a rice glutelin gene, a rice lactate dehydrogenase gene and a rice beta-tubulin gene are disclosed in U.S. published patent application 2002/0192813 A1.

[0031] Constructs and vectors may also include a transit peptide for targeting of a gene target to a plant organelle, particularly to a chloroplast, leucoplast or other plastid

organelle. The use of chloroplast transit peptides is disclosed in U.S. Patents 5,188,642 and 5,728,925.

[0032] The plants of this invention can be further enhanced with stacked traits, e.g., a crop having an enhanced agronomic trait resulting from expression of DNA disclosed herein, in combination with herbicide and/or pest resistance traits. For example, genes of the current invention can be stacked with other traits of agronomic interest, such as a trait providing herbicide resistance, or insect resistance, such as using a gene from *Bacillus thuringiensis* to provide resistance against lepidopteran, coleopteran, homopteran, hemiopteran, and other insects. Herbicides for which resistance is useful in a plant include glyphosate herbicides, dicamba herbicides, phosphinothricin herbicides, oxynil herbicides, imidazolinone herbicides, dinitroaniline herbicides, pyridine herbicides, sulfonylurea herbicides, bialaphos herbicides, sulfonamide herbicides and glufosinate herbicides. Persons of ordinary skill in the art are enabled in providing stacked traits by reference to U.S. 2003/0106096A1 and 2002/0112260A1 and US Patents 5,034,322; 5,776,760; 6,107,549 and 6,376,754 and to insect/nematode/virus resistance by reference to U.S. Patents 5,250,515; 5,880,275; 6,506,599; 5,986,175 and U.S. 2003/0150017 A1.

Plant Cell Transformation Methods

[0033] Numerous methods for transforming plant cells with recombinant DNA are known in the art and may be used in the present invention. Two commonly used methods for plant transformation are *Agrobacterium*-mediated transformation and microprojectile bombardment. Microprojectile bombardment methods are illustrated in U.S. Patents 5,015,580 (soybean); 5,550,318 (corn); 5,538,880 (corn); 5,914,451 (soybean); 6,160,208 (corn); 6,399,861 (corn) and 6,153,812 (wheat) and *Agrobacterium*-mediated transformation is described in U.S. Patents 5,159,135 (cotton); 5,824,877 (soybean); 5,591,616 (corn); and 6,384,301 (soybean), all of which are incorporated herein by reference. For *Agrobacterium tumefaciens* based plant transformation system, additional elements present on transformation constructs will include T-DNA left and right border sequences to facilitate incorporation of the recombinant polynucleotide into the plant genome.

[0034] In general it is useful to introduce recombinant DNA randomly, i.e. at a non-specific location, in the genome of a target plant line. In special cases it may be useful to target recombinant DNA insertion in order to achieve site-specific integration, for example to replace an existing gene in the genome, to use an existing promoter in the plant genome, or to insert a recombinant polynucleotide at a predetermined site known to be active for gene expression. Several site specific recombination systems exist which are known to function implants include cre-lox as disclosed in U.S. Patent 4,959,317 and FLP-FRT as disclosed in U.S. Patent 5,527,695.

[0035] Transformation methods of this invention are preferably practiced in tissue culture on media and in a controlled environment. "Media" refers to the numerous nutrient mixtures that are used to grow cells *in vitro*, that is, outside of the intact living organism. Recipient cell targets include, but are not limited to, meristem cells, callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells. It is contemplated that any cell from which a fertile plant may be regenerated is useful as a recipient cell. Callus may be initiated from tissue sources including, but not limited to, immature embryos, seedling apical meristems, microspores and the like. Cells capable of proliferating as callus are also recipient cells for genetic transformation. Practical transformation methods and materials for making transgenic plants of this invention, for example various media and recipient target cells, transformation of immature embryo cells and subsequent regeneration of fertile transgenic plants are disclosed in U.S. Patents 6,194,636 and 6,232,526, which are incorporated herein by reference.

[0036] The seeds of transgenic plants can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plants line for selection of plants having an enhanced trait. In addition to direct transformation of a plant with a recombinant DNA, transgenic plants can be prepared by crossing a first plant having a recombinant DNA with a second plant lacking the DNA. For example, recombinant DNA can be introduced into first plant line that is amenable to transformation to produce a transgenic plant which can be crossed with a second plant line to introgress the recombinant DNA into the second plant line. A transgenic plant with recombinant DNA providing an enhanced trait, e.g. enhanced yield, can be crossed with transgenic plant line having other recombinant DNA that confers

another trait, for example herbicide resistance or pest resistance, to produce progeny plants having recombinant DNA that confers both traits. Typically, in such breeding for combining traits the transgenic plant donating the additional trait is a male line and the transgenic plant carrying the base traits is the female line. The progeny of this cross will segregate such that some of the plants will carry the DNA for both parental traits and some will carry DNA for one parental trait; such plants can be identified by markers associated with parental recombinant DNA, e.g. marker identification by analysis for recombinant DNA or, in the case where a selectable marker is linked to the recombinant, by application of the selecting agent such as a herbicide for use with a herbicide tolerance marker, or by selection for the enhanced trait. Progeny plants carrying DNA for both parental traits can be crossed back into the female parent line multiple times, for example usually 6 to 8 generations, to produce a progeny plant with substantially the same genotype as one original transgenic parental line but for the recombinant DNA of the other transgenic parental line.

[0037] In the practice of transformation DNA is typically introduced into only a small percentage of target plant cells in any one transformation experiment. Marker genes are used to provide an efficient system for identification of those cells that are stably transformed by receiving and integrating a transgenic DNA construct into their genomes. Preferred marker genes provide selective markers which confer resistance to a selective agent, such as an antibiotic or herbicide. Any of the herbicides to which plants of this invention may be resistant are useful agents for selective markers. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene is integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA. Commonly used selective marker genes include those conferring resistance to antibiotics such as kanamycin and paromomycin (*nptII*), hygromycin B (*aph IV*) and gentamycin (*aac3* and *aac*C4) or resistance to herbicides such as glufosinate (*bar* or *pat*) and glyphosate (*aroA* or EPSPS). Examples of such selectable are illustrated in U.S. Patents 5,550,318; 5,633,435; 5,780,708 and 6,118,047. Selectable markers which provide an ability to visually identify transformants can also be employed, for example, a gene expressing a colored or

fluorescent protein such as a luciferase or green fluorescent protein (GFP) or a gene expressing a *beta*-glucuronidase or *uid*A gene (GUS) for which various chromogenic substrates are known.

[0038] Plant cells that survive exposure to the selective agent, or plant cells that have been scored positive in a screening assay, may be cultured in regeneration media and allowed to mature into plants. Developing plantlets regenerated from transformed plant cells can be transferred to plant growth mix, and hardened off, for example, in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO_2, and 25-250 microeinsteins $m^{-2} s^{-1}$ of light, prior to transfer to a greenhouse or growth chamber for maturation. Plants are regenerated from about 6 weeks to 10 months after a transformant is identified, depending on the initial tissue. Plants may be pollinated using conventional plant breeding methods known to those of skill in the art and seed produced, for example self-pollination is commonly used with transgenic corn. The regenerated transformed plant or its progeny seed or plants can be tested for expression of the recombinant DNA and selected for the presence of enhanced agronomic trait.

Transgenic Plants and Seeds

[0039] Transgenic plants derived from the plant cells of this invention are grown to generate transgenic plants having an enhanced trait as compared to a control plant and produce transgenic seed and haploid pollen of this invention. Such plants with enhanced traits are identified by selection of transformed plants or progeny seed for the enhanced trait. For efficiency a selection method is designed to evaluate multiple transgenic plants (events) including the recombinant DNA, for example multiple plants from 2 to 20 or more transgenic events. Transgenic plants grown from transgenic seed provided herein demonstrate improved agronomic traits that contribute to increased yield or enhanced water deficit tolerance or both.

[0040] Not all transgenic events will be in transgenic plant cells that provide plants and seeds with an enhanced or desired trait depending on factors, such as location and integrity of the recombinant DNA, copy number, unintended insertion of other DNA, etc. As a result transgenic plant cells of this invention are identified by screening transformed progeny plants for enhanced water deficit stress tolerance and yield. For

efficiency a screening program is designed to evaluate multiple transgenic plants preferably with a single copy of the recombinant DNA from 2 or more transgenic events.

[0041] The following examples are included to demonstrate embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention, therefore all matter set forth or shown in the accompanying drawings and examples is to be interpreted as illustrative and not in a limiting sense.

Example 1

[0042] This example describes construction of plant expression vectors used for transforming plant cells useful in the various aspects of the invention. Plasmid maps of such DNA constructs are illustrated in Figures 1 and 2, where the plasmid of Figure 1 is used for transforming monocot plants such as corn and the plasmid of Figure 2 is used for transforming dicot plants such as soybean. Each plasmid contains a NF-YB expression cassette and a glyphosate herbicide resistance expression cassette within *Agrobacterium tumefaciens* T-DNA borders identified as LB and RB respectively. Each plasmid also contains origins of replication and repressor elements for replication in cells (oriV, rop and oriColE) and a spectinomycin/streptomycin bactericidal selectable marker (SPC/STR).

[0043] With particular reference to Figure 1 plasmid pMON82754 contains between left and right Agrobacterium T-DNA borders (LB and RB) recombinant DNA constructs comprising an NF-YB protein expression cassette and a glyphosate resistance expression cassette. The NF-YB expression cassette has a promoter element (SEQ ID NO:8) which comprises a corn bundle sheath enhanced promoter and a transcription enhancing intron from a corn heat shock protein 70 gene followed by the DNA encoding a corn NF-YB protein (SEQ ID NO:44) and a 3' element from *Agrobacterium tumefaciens* transcript 7. The glyphosate resistance expression cassette comprises a rice

actin 1 promoter, leader and intron operably linked to a DNA encoding a chloroplast transit peptide from an *Arabidopsis thaliana* EPSPS gene and DNA encoding an EPSPS from an *A. tumefaciens* gene (CP4) and a 3' element from an *A. tumefaciens* nopaline synthase gene. Other plasmids are prepared in which the promoter element and DNA encoding the NF-YB element are replaced with each of the promoter elements identified in Table 1 and each of the DNA encoding NF-YB protein elements identified in Table 2. Thus, separate plasmids for transforming monocot plant cells have recombinant DNA constructs for expressing an NF-YB protein where each promoter identified in Table 1 is operably linked to each of the DNAs encoding an NF-YB protein identified in Table 2. Monocot plant cells from corn, switchgrass, rice, and sugarcane are transformed with each of the plasmids producing multiple transgenic events of each recombinant DNA construct; and transgenic plants are regenerated and grown to produce transgenic seed or propagule (in the case of sugarcane) for each of the transgenic events.

[0044] With reference to Figure 2, plasmid pMON63796 contains a recombinant DNA construct for expressing NF-YB protein with an enhanced CaMV35S promoter (SEQ ID NO:1) operably linked to DNA encoding the native *Arabidopsis thaliana* NF-YB protein identified as G481 (SEQ ID NO:53). The plasmid is used to produce transgenic dicot cells in which the *Arabidopsis* NF-YB protein is ubiquitously expressed by the promoter. Other plasmids are prepared in which the promoter element is replaced with each of the promoter elements identified in Table 1 and the DNA encoding the NF-YB element is replaced with each of the DNA encoding NF-YB protein elements identified in Table 2. Thus, separate plasmids for transforming dicot plant cells have recombinant DNA constructs for expressing an NF-YB protein where each promoter identified in Table 1 is operably linked to each DNA encoding NF-YB protein identified in Table 2. Canola, alfalfa, cotton and soybean plant cells are transformed with each of the plasmids producing multiple transgenic events of each recombinant DNA construct; and transgenic plants are regenerated and grown to produce transgenic seed for each of the transgenic events.

[0045] Many transgenic events which survive to fertile transgenic plants that produce seeds and progeny plants will not exhibit the traits of water deficit stress tolerance and enhanced yield. Screening of progeny transgenic plants is necessary to

identify a transgenic plant cell of this invention. Transgenic plants having enhanced water deficit tolerance are identified from populations of plants transformed as described herein by evaluating the trait in a variety of water deficit assays. More specifically, after transformation transgenic plants are propagated to produce seed or propagules and homozygous progeny plants are identified and screened for water deficit tolerance (e.g. using methods described below) to identify plants that produce seed of this invention.

[0046] The plants of this invention are screened for water deficit tolerance as compared to control plants (tested as inbreds or hybrids) by a high-throughput method of greenhouse screening following water withholding, called "drought treatment". For example, the greenhouse screen for transgenic corn plants for water use efficiency measures changes in plant growth rate, e.g., at least a 10% improvement, in height and biomass during a vegetative drought treatment, as compared to control plants. The hydration status of the shoot tissues following the drought is also measured. Shoot Initial Height (SIH) is plant height after 3 weeks of growth under optimum conditions. Shoot Wilt Height (SWH) is plant height at the end of a 6 day drought. Time course experiments have shown that at about 3 days of drought treatment, wild type corn plants basically stop growing and begin to wilt. Thus a transgenic corn plant with improved water use efficiency will continue to grow (possibly to a lesser extent than with water) and thereby end up as a significantly taller plant at the end of a drought experiment. Shoot Wilt Mass (SWM) is the amount of wet and dry matter in the shoot (plant separated from root ball at the soil line) at the end of the drought; SDM is measure after 2 to 3 weeks in a drying chamber. Shoot Turgid mass (STM) is the SWM plus the mass of the water that is transported into plant tissues in 3 days of soaking in 40 degree Celsius water in the dark. Experiments have shown that most of the water is pulled up in 24 hours but it takes 2 more days before additional increase becomes insignificant. STM-SWM is indicative of water use efficiency in plants where recovery from stress is more important than stress tolerance per se. Relative water content (RWC) is a measurement of how much (%) of the plant is water at harvest. RWC = (SWM-SDM)/(STM-SDM)*100. Fully watered corn plants are about 98% RWC. Typically, in a wilt screen the plants are about 60% RWC. Plants with higher RWC at the end of a drought are considered to be healthier plants and more fit for post-drought recovery and growth.

Relative Growth Rate (RGR) is calculated for each shoot using the formula RGR = (SWH-SIH)/((SWH+SIH)/2)*100. Similar screening following a drought treatment is done for transgenic canola, cotton and soybean plants.

[0047] Events of transgenic corn plants expressing a modified NF-YB protein from DNA of SEQ ID NO:29-43 show improved water deficit stress tolerance as compared to wild type control plants and transgenic control plants expressing a natural NF-YB protein.

[0048] Events of transgenic corn plants expressing a native NF-YB protein from DNA of SEQ ID NO: 44 or 45 operably linked to a promoter selected from SEQ ID NO: 1-27 show improved water deficit stress tolerance as compared to wild type control plants.

Example 2

[0049] This example describes yield analysis results for transgenic plants disclosed in US 2005/0022266 A1. The disclosed plants comprise a recombinant transcription unit comprising a low level-expressing rice actin promoter operably linked to DNA coding for an NF-YB (SEQ ID NO:28) protein followed by a transcription unit comprising a CaMV 35S promoter operably linked to DNA coding for the nptII marker (pMON73605).

[0050] Table 3A demonstrates that these plants exhibit enhanced yield (bu/acre) under water deficit stress, as evidenced by two consecutive years of testing.

Table 3A - Improved Yield Under Water Deficit Stress

pMON73605 – rice actin 1 promoter:NF-YB2 (expression enhanced by cis elements)				
Event	**Year 1**		**Year 2**	
	Water Deficit Stress Yield Delta	**Water Deficit Stress p-value**	**Water Deficit Stress Yield Delta**	**Water Deficit Stress p-value**
ZM_M23837	13.7	.012	16.6	0.13
ZM_M25520	31.2	0.00	15.9	0.13
ZM_M24207	54.8	<.001	16.9	0.11

[0051] Table 3B presents yield results (bu/acre) under sufficient water conditions and demonstrates that the yield of pMON73605 transgenic plants under sufficient water conditions is significantly decreased.

Table 3B – Reduced Yield Under Sufficient Water Conditions

Event	Year 1		Year 2	
	Sufficient Water Yield Delta	Sufficient Water p-value	Sufficient Water Yield Delta	Sufficient Water p-value
ZM_M23837	-54.9	<.001	-23	<.001
ZM_M25520	-40.3	<.001	-28	<.001
ZM_M24207	-51.3	<.001	-35	<.001

NFY B protein levels determined for these transgenic events are provided in Table 3C below.

Table 3C NF-YB2 protein levels

	V12 to VT leaf protein
Event	pg NF-YB2/microgram total protein
ZM_M23837	61.4
ZM_M25520	51.8
ZM_M24207	61.0

[0052] The CaMV 35S promoter in this construct is able to enhance the otherwise low expression of the nearby rice actin promoter resulting in the production of greater than 40 picograms of NF-YB2 protein per microgram of total protein in the plant leaf tissue. The high level of protein in the transgenic plants results in a reduction in yield when the plants are grown under water sufficient conditions. In contrast, enhancerless rice actin/NFYB expression constructs (pMON82452 and pMON82453) are described below which produce less than 20 pg NF-YB2/microgram total leaf protein and transgenic plants having enhanced yield under both water deficit stress conditions and sufficient water conditions are described.

Example 3

[0053] Transgenic corn plants prepared as described in Example 1 comprising DNA constructs stably inserted in the chromosome and expressing an NF-YB protein under the control of promoters shown in Table 4 were evaluated for yield under water deficit stress and sufficient conditions.

Table 4 Promoter::NF-YB Constructs in Transgenic Corn Plants

Promoter	Promoter SEQ ID	Protein	Vector
Enhanced CaMV 35S	SEQ ID NO:2	NFB2	PMON84654
Rubisco activase	SEQ ID NO:8	NFB2	PMON82754
Rice tubulin	SEQ ID NO:13	NFB2a	pMON82753
Rice tubulin	SEQ ID NO:13	NFB2	pMON82752
rab17	SEQ ID NO:9	NFB2	PMON82454
Enhancerless rice actin	SEQ ID NO:15	NFB2a	PMON82453
Enhancerless rice actin	SEQ ID NO:14	NFB2	PMON82452
p326	SEQ ID NO:18	NFB2	PMON78305
FDA/PPDK	SEQ ID NO:27	NFB2	PMON78304
PPDK	SEQ ID NO:10	NFB2	PMON78303
CaMV 35S	SEQ ID NO:3	NFB2	PMON73611
NAS	SEQ ID NO:16	NFB2	PMON73610

[0054] These transgenic corn plants were analyzed for yield under water-deficit stress conditions, for yield under water sufficiency conditions and for amounts of NF-YB protein expressed in leaf tissue.

[0055] Homozygous inbred corn plants with recombinant DNA as described in Example 1 were crossed with compatible tester lines to produce hybrid seed. The resulting seed was advanced to replicated yield trials in geographical regions where corn is conventionally grown, e.g. in the states of Iowa, Illinois, Kansas and California. In some trials, field water content was controlled by irrigation, while other trials relied on natural rainfall. Transgenic events advanced to this study were pre-selected to be single copy for the selectable marker associated with the transgene. Control and transgenic events were planted at the same plant density and replication. Field management of plant pest, weeds, tillage and fertilization was consistent with geography specific practices.

[0056] In irrigated fields, the transgenic and control plants were irrigated to be within field water-holding capacity until V10 corn leaf stage. Irrigation water was delivered via drip irrigation or overhead linear irrigation. To provide water deficit stress conditions, once the corn plants reached the V10 leaf stage, water was allowed to be limiting until plants demonstrated significant AM leaf rolling for 2 consecutive days. The duration of this water regime spanned the V10 leaf through the R2 reproductive stage. Once the crop reached the R2 developmental stage, watering was resumed to full recovery through the remaining growing season.

[0057] Once the corn crop reached physiological maturity, i.e. 10-25% grain moisture, plots were harvested. Resulting grain yield was normalized to 15.5% moisture and expressed in terms of bushels/acre.

[0058] Yield data analysis was performed for water deficit stress and sufficient water conditions. The yields were analyzed in several ways. One approach involved the use of statistical cluster analysis to select groups of locations having similar environmental characteristics pertaining to drought. The three variables used to form clusters of similar locations were: the average daily high temperature during the thirty days before and thirty days after flowering; the average difference between cumulative precipitation and applied water versus evapotranspiration during the same sixty-day period, weighted by the proximity to flowering using the standard normal distribution to define weights; and the average yield of a control pedigree at the location. Another analysis of variance model was then used to analyze yields, with fixed effects for clusters and random effects for the locations nested within their corresponding clusters.

[0059] The yield data analysis compared the yields of all events from a single construct as compared to corresponding control plots. Events were also compared to a corresponding control for event level analysis. Results of the yield analysis (Yield Deltas shown as bu/ac) are reported in Tables 5A to 5M.

Table 5A

pMON82753 – rice alpha tubulin promoter SEQ ID NO 13:NF-YB2a				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_M86067	4.5106	0.266	16.0421	0.011
ZM_M83476	-6.6879	0.116	11.763	0.08
ZM_M84797	-3.6472	0.38	11.7199	0.064
ZM_M83475	2.8742	0.478	5.4529	0.364
ZM_M84738	2.1377	0.624	1.7943	0.789
ZM_M83470	-2.3375	0.862	1.088	0.871
ZM_M84105	-15.0532	<.001	0.4693	0.944
ZM_M84086	1.4321	0.736	-2.1618	0.78
ZM_M83465	0.3697	0.927	-4.4721	0.456
ZM_M86087	-11.0371	0.006	-5.8471	0.33
ZM_M84741	-11.3175	0.006	-7.9079	0.211
ZM_M83478	-5.2598	0.195	-8.0171	0.182
ZM_M86065	-8.7812	0.355	-8.8923	0.215

Table 5B

pMON82454 – rab17 promoter SEQ ID NO 9:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S112714	0.3879	0.924	-12.0102	0.036
ZM_S112682	0.7015	0.863	-5.8292	0.357
ZM_S110587	-3.2144	0.428	-4.6313	0.441
ZM_S112667	-7.003	0.084	-3.7511	0.512
ZM_S111896	-3.5696	0.401	-1.992	0.728
ZM_S112713	10.2417	0.445	0.09028	0.989
ZM_S110594	-4.3189	0.287	0.4137	0.945
ZM_S112696	-6.3222	0.128	0.4887	0.935
ZM_S112654	-8.3365	0.045	0.867	0.88
ZM_S112701	-5.4576	0.178	0.9761	0.865
ZM_S112657	-0.4083	0.976	1.0792	0.865

Table 5C

pMON82754 – corn bundle sheath promoter SEQ ID NO 8:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S110144	-6.9826	0.085	13.5813	0.024
ZM_S110890	-4.9583	0.522	10.6975	0.111
ZM_S110843	-2.2576	0.578	4.5413	0.449
ZM_S110837	-3.3767	0.427	3.8793	0.54
ZM_S110106	-12.4485	0.002	1.0213	0.865
ZM_S110893	-7.8212	0.054	0.2913	0.961
ZM_S111476	-3.6553	0.367	-0.2487	0.967
ZM_S110134	-10.1762	0.014	-3.3787	0.574
ZM_S110873	-0.419	0.92	-6.0041	0.343
ZM_S110119	-6.1598	0.129	-14.7387	0.014

Table 5D

pMON84654 – enhancedCaMV35S promoter SEQ ID NO2:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S119096	-46.7705	<.001	-0.09792	0.988
ZM_S117108	-43.9468	<.001	-11.8074	0.062
ZM_S119061	-37.014	<.001	-11.8317	0.049
ZM_S119030	-44.4451	<.001	-12.8967	0.032
ZM_S119088	-48.1538	<.001	-13.1315	0.038
ZM_S119034	-48.0195	<.001	-13.5567	0.024
ZM_S119047	-52.5443	<.001	-13.8889	0.028
ZM_S117101	-41.4371	<.001	-14.4537	0.022
ZM_S117341	-55.5489	<.001	-15.3574	0.015
ZM_S119099	-43.7741	<.001	-15.7417	0.009
ZM_S119033	-45.9945	<.001	-18.9417	0.002
ZM_S119077	-31.7358	<.001	-20.6117	<.001
ZM_S117346	-15.6325	0.099		

Table 5E

pMON82752 – rice alpha tubulin promoter SEQ ID NO13:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_M83933	-8.83	0.033	14.363	0.017
ZM_M82773	0.4403	0.916	10.4845	0.067
ZM_M84408	-0.8068	0.853	7.0527	0.218
ZM_M82770	-14.2708	0.193	5.5578	0.438
ZM_M82769	-2.0203	0.763	5.2828	0.461
ZM_M84389	-1.4877	0.714	3.9209	0.494
ZM_M82855	-35.2312	0.009	3.4116	0.59
ZM_M83306	-1.217	0.832	0.9614	0.893
ZM_M84393	0.8677	0.834	-5.182	0.388
ZM_M83321	3.2487	0.423	-5.7246	0.317
ZM_M82772	-0.8763	0.829	-5.952	0.322
ZM_M85712	-10.5414	0.022	-6.5328	0.302

Table 5F

pMON82453 - rice actin1 promoter SEQ ID NO15:NF-YB2a				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_M87052	-3.9922	0.401	-7.594	0.258
ZM_M88601	6.2671	0.131	-4.0602	0.499
ZM_M87033	1.4629	0.718	-0.9602	0.873
ZM_M88602	5.8765	0.147	-0.8908	0.894
ZM_M88129	0.3765	0.926	0.01482	0.998
ZM_M87027	2.2129	0.585	0.3398	0.955
ZM_M87051	-4.2189	0.298	0.6598	0.912
ZM_M87036	-1.8401	0.657	4.072	0.52
ZM_M87378	0.797	0.844	5.772	0.362
ZM_M88595	6.2552	0.132	6.5848	0.273
ZM_M87049	4.1652	0.304	7.6698	0.201
ZM_M87335	0.4311	0.915	9.5915	0.13

Table 5G

pMON82452 - rice actin1 promoter SEQ ID NO 14:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_M87949	5.1553	0.215	6.5758	0.299
ZM_M85725	-9.5685	0.021	1.1316	0.851
ZM_M87438	-9.3518	0.021	0.7814	0.902
ZM_M87019	0.5323	0.896	-0.7982	0.9
ZM_M87010	0.6017	0.885	-1.5234	0.8
ZM_M87952	-2.5268	0.533	-2.5019	0.693
ZM_M85731	-1.7604	0.759	-3.2605	0.627
ZM_M87441	1.9918	0.64	-4.7293	0.481
ZM_M85734	-3.2154	0.428	-4.8284	0.421
ZM_M87937	0.03685	0.993	-6.8934	0.251
ZM_M87427	1.0596	0.794	-7.5684	0.208
ZM_M87000	-0.4518	0.911	-8.2884	0.168
ZM_M87936	-6.7261	0.202	-8.3605	0.213

Table 5H

pMON78305 – P326 promoter SEQ ID 18:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S121122	0.8762	0.833	4.1907	0.508
ZM_S121068	-0.7	0.863	4.0241	0.525
ZM_S121130	-0.05682	0.989	3.7963	0.549
ZM_S121124	2.6568	0.512	2.0417	0.734
ZM_S121091	5	0.218	-3.5593	0.574
ZM_S121092	-0.1214	0.977	-5.4083	0.368
ZM_S121070	-2.819	0.497	-5.75	0.364
ZM_S121096	2.6024	0.531	-7.7981	0.218
ZM_S121064	3.5205	0.385	-11.0033	0.067
ZM_S121123	4.4341	0.274	-11.7033	0.051

Table 5I

pMON78304 – FDA/PPDK promoter SEQ ID NO 27:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S120150	-15.8444	<.001	-0.53	0.93
ZM_S120028	-14.4106	<.001	-0.9697	0.866
ZM_S119399	-8.5762	0.039	-6.0833	0.288
ZM_S119395	-9.2742	0.022	-7.9389	0.21
ZM_S120146	-15.7765	<.001	-9.3407	0.14
ZM_S120046	-16.665	<.001	-12.3067	0.04

Table 5J

pMON78303 – PPDK promoter SEQ ID 10:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S114670	-5.0583	0.212	9.3727	0.139
ZM_S114672	-8.8856	0.028	5.9254	0.324
ZM_S116983	-18.1152	<.001	5.8404	0.331
ZM_S115592	-12.9152	0.001	5.4604	0.363
ZM_S115605			5.2875	0.693
ZM_S115597	-10.8538	0.007	5.1095	0.372
ZM_S115611	-11.1083	0.006	4.1304	0.492
ZM_S115590	-14.397	<.001	-0.8846	0.883
ZM_S117062	-13.6379	<.001	-5.3746	0.371
ZM_S114676	-9.1311	0.024	-6.0133	0.294
ZM_S114678	-14.2968	<.001	-6.8546	0.254
ZM_S117016	-7.45	0.496	-14.662	0.029

Table 5K

pMON73611 - CaMV35S promoter SEQ ID NO 3:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S115348	-12.6042	0.25	-0.1982	0.978
ZM_S114565	-23.2198	<.001	-0.6462	0.923
ZM_S114577	-16.5312	<.001	-2.6359	0.645
ZM_S114556	-17.9972	<.001	-4.072	0.498
ZM_S115373	-20.5903	<.001	-4.7313	0.409
ZM_S115260	-26.2934	<.001	-4.7775	0.476
ZM_S114535	-19.7153	<.001	-6.122	0.308
ZM_S114591	-25.0631	<.001	-8.4177	0.142
ZM_S115266	-14.0919	<.001	-12.0466	0.057
ZM_S115340	-17.9381	<.001	-15.3295	0.011
ZM_S115350	-21.8187	0.104	-15.4681	0.021
ZM_S115261	-21.3938	0.111	-20.2605	0.001

Table 5L

pMON73610 – corn root promoter SEQ ID NO 16:NF-YB2				
Event	Sufficient Water Yield Delta	Sufficient Water p-value	Water Deficit Stress Yield Delta	Water Deficit Stress p-value
ZM_S115519	3.9933	0.349	8.0867	0.297
ZM_S115721	1.7023	0.675	7.19	0.283
ZM_S114691	6.5114	0.108	5.4689	0.388
ZM_S115769	8.8727	0.029	5.242	0.383
ZM_S114480	4.2227	0.299	4.172	0.487
ZM_S115567	-2.3	0.716	4.091	0.569
ZM_S117076	1.7811	0.717	2.6267	0.714
ZM_S115703	-2.05	0.613	-0.6922	0.913

Table 5M

pMON73605 – rice actin 1 promoter:NF-YB2 (expression enhanced by cis elements)				
Event	**Sufficient Water Yield Delta**	**Sufficient Water p-value**	**Water Deficit Stress Yield Delta**	**Water Deficit Stress p-value**
ZM_M23837	-23.1583	<.001	-20.7881	<.001
ZM_M24207	-21.8297	<.001	-12.3592	0.057
ZM_M25520	-25.9283	<.001	-15.8775	0.023
ZM_M26961	-23.7417	<.001	-10.1582	0.106
ZM_M26962	-24.8995	<.001	-12.6188	0.052

[0060] To correlate expression of NF-YB protein with water-deficit tolerance and yield under water deficit and optimal water conditions, NF-YB2 protein was measured in the transgenic plants by standard ELISA techniques using polyclonal antibodies raised in rabbit. NF-YB2 protein level is reported as "picograms of NF-YB2 protein per microgram of total protein" and includes both native and exogenous NF-YB2 protein. Total protein was measured using the Bradford protein assay (Bio-Rad, Hercules, CA). Background levels of NF-YB2 protein (pg NF-YB2 / μg total protein) in each of the tissues measured are as follows:

V3 leaf	3.5
V12 leaf	6
Root	2.5
Silk	5
Tassel	3.2
Kernel	9.1
Immature cob	22.6

[0061] Results of analysis of protein levels in various tissues and at various stages of development are reported in Tables 6A and 6B as the average of multiple events for each construct.

Table 6A

			pg NF-YB2 / µg total protein				
Construct	Promoter sequence	Yield table	Leaf (V3)	Leaf (V12)	Leaf (V15)	Leaf (VT-R1)	average
PMON84654	2	5D	107.0	121.9	195.3	136.4	144.4
PMON78303	10	5J	71.6	83.1	141.5	112.1	106.9
PMON73611	3	5K	66.0	67.8	127.1	79.6	88.8
PMON78304	27	5I	49.1	56.2	108.7	73.4	75.1
PMON73605	15	5M	39.5	57.7	79.6	54.2	62.2
PMON82754	8	5C	36.5	35.4	64.5	32.6	43.3
PMON78305	18	5H	7.8	5.1	5.3	4.7	5.2
PMON82752	13	5E	6.6	4.9	6.5	5.5	5.6
PMON82452	14	5G	6.0	7.8	10.5	8.7	8.8
PMON73610	16	5L	5.2	3.9	4.5	3.5	4.0
PMON82454	9	5B	1.1	9.2	11.4	5.5	8.5

Table 6B

			pg NF-YB2 / µg total protein				
Construct	Promoter sequence	yield table	Root	Silk	Tassel	Kernel	Immature Cob
PMON84654	2	5D	4.6	9.6	33.6	33.2	149.6
PMON78303	10	5J	4.9	7.0	12.3	18.4	26.8
PMON73611	3	5K	1.8	18.4	42.9	32.4	59.4
PMON78304	27	5I	3.3	2.9	50.7	21.4	28.3
PMON73605	15	5M	15.6	38.0	20.8	22.2	60.7
PMON82754	8	5C	3.2	6.9	11.5	19.3	44.4
PMON78305	18	5H	1.7	5.4	10.3	15.8	40.1
PMON82752	13	5E	4.5	4.3	9.8	28.0	106.2
PMON82452	14	5G	3.1	3.5	66.8	31.6	38.8
PMON73610	16	5L	3.5	21.6	7.9	33.5	36.7
PMON82454	9	5B	3.9	2.8	4.7	19.6	32.0

[0062] Results of the analysis of data for yield under water-deficit stress conditions, yield under water sufficiency conditions and amounts of NF-YB protein expressed in leaf tissue (corrected to substract background level of NF-YB protein

produced from the native DNA) for individual events is presented in Table 7 (as compared to the aggregated construct level data presented in Table 6A and 6B). The data demonstrate an inverse correlation between the level of NF-YB protein expressed and enhanced yield under both water-deficit stress conditions and sufficient water conditions. When yield (bu/acre) is plotted against NF-YB levels, events that show enhanced yield under water-deficit stress conditions, also contained low levels of NF-YB (up to 40 picograms of NF-YB2 protein per microgram of total protein in the plant leaf tissue) (Fig. 3). Similarly, events that show enhanced yield (Yield Deltas shown as bu/acre) under water sufficient conditions also contained low levels of NF-YB (up to 40 picograms of NF-YB2 protein per microgram of total protein in the plant leaf tissue) (Fig. 4). An especially useful embodiment for enhanced yield in corn under a wide range of available water conditions provides 0.1 to 11 pg NFB2/ug total protein. Particular examples are ZM_M87949 (8.0 pg NFB2/ug total protein; +6.6 bu/acre yield increase under water-deficit stress; +5.2 bu/acre yield increase under water sufficiency) and ZM_M88595 (7.5 pg NFB2/ug total protein; +6.6 bu/acre yield increase under water-deficit stress; +6.3 bu/acre yield increase under water sufficiency).

Table 7. Yield and Protein Data from Corn Containing Promoter::NF-YB Constructs

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
Enhanced CaMV 35S	PMON84654	ZM_S117101	181.5	-41.4	-14.5
Enhanced CaMV 35S	PMON84654	ZM_S117341	177.3	-55.5	-15.4
Enhanced CaMV 35S	PMON84654	ZM_S119096	170.7	-46.8	-0.1
Enhanced CaMV 35S	PMON84654	ZM_S119034	168.1	-48.0	-13.6
Enhanced CaMV 35S	PMON84654	ZM_S119033	165.6	-46.0	-18.9
Enhanced CaMV 35S	PMON84654	ZM_S119061	164.8	-37.0	-11.8

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
Enhanced CaMV 35S	PMON84654	ZM_S117108	158.7	-43.9	-11.8
Enhanced CaMV 35S	PMON84654	ZM_S119088	157.2	-48.2	-13.1
Enhanced CaMV 35S	PMON84654	ZM_S119099	143.7	-43.8	-15.7
Enhanced CaMV 35S	PMON84654	ZM_S119030	139.3	-44.4	-12.9
Enhanced CaMV 35S	PMON84654	ZM_S119047	136.0	-52.5	-13.9
PPDK	PMON78303	ZM_S115590	123.8	-14.4	-0.9
PPDK	PMON78303	ZM_S114678	121.0	-14.3	-6.9
CaMV 35S	PMON73611	ZM_S114565	117.1	-23.2	-0.6
PPDK	PMON78303	ZM_S115592	110.8	-12.9	5.5
Enhanced CaMV 35S	PMON84654	ZM_S119077	110.4	-31.7	-20.6
PPDK	PMON78303	ZM_S115597	107.4	-10.9	5.1
PPDK	PMON78303	ZM_S115611	107.1	-11.1	4.1
PPDK	PMON78303	ZM_S114670	106.4	-5.1	9.4
PPDK	PMON78303	ZM_S114672	105.4	-8.9	5.9
CaMV 35S	PMON73611	ZM_S114591	104.7	-25.1	-8.4
PPDK	PMON78303	ZM_S116983	104.6	-18.1	5.8
Enhanced CaMV 35S	PMON84654	ZM_S117346	104.3	-15.6	Not determined
PPDK	PMON78303	ZM_S117062	102.3	-13.6	-5.4
CaMV 35S	PMON73611	ZM_S115340	102.1	-17.9	-15.3
CaMV 35S	PMON73611	ZM_S115260	100.9	-26.3	-4.8
PPDK	PMON78303	ZM_S115605	100.8	Not determined	5.3
CaMV 35S	PMON73611	ZM_S115373	97.2	-20.6	-4.7
CaMV 35S	PMON73611	ZM_S114535	92.8	-19.7	-6.1
Rubisco activase	PMON82754	ZM_S110893	86.9	-7.8	0.3
CaMV 35S	PMON73611	ZM_S114577	86.8	-16.5	-2.6
PPDK	PMON78303	ZM_S114676	83.0	-9.1	-6.0
FDA/PPDK	PMON78304	ZM_S120046	76.7	-16.7	-12.3
FDA/PPDK	PMON78304	ZM_S120146	75.8	-15.8	-9.3
FDA/PPDK	PMON78304	ZM_S120028	75.1	-14.4	-1.0

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
CaMV 35S	PMON73611	ZM_S115261	74.7	-21.4	-20.3
PPDK	PMON78303	ZM_S117016	74.4	-7.5	-14.7
FDA/PPDK	PMON78304	ZM_S119395	72.2	-9.3	-7.9
CaMV 35S	PMON73611	ZM_S115266	72.1	-14.1	-12.0
CaMV 35S	PMON73611	ZM_S115350	71.7	-21.8	-15.5
FDA/PPDK	PMON78304	ZM_S120150	70.8	-15.8	-0.5
CaMV 35S	PMON73611	ZM_S115348	69.6	-12.6	-0.2
FDA/PPDK	PMON78304	ZM_S119399	64.9	-8.6	-6.1
CaMV 35S	PMON73611	ZM_S114556	61.3	-18.0	-4.1
Rubisco activase	PMON82754	ZM_S110843	53.8	-2.3	4.5
Rubisco activase	PMON82754	ZM_S110119	48.7	-6.2	-14.7
Rubisco activase	PMON82754	ZM_S110134	46.6	-10.2	-3.4
Rubisco activase	PMON82754	ZM_S110106	44.5	-12.4	1.0
Rubisco activase	PMON82754	ZM_S110144	43.4	-7.0	13.6
Rubisco activase	PMON82754	ZM_S110890	42.0	-5.0	10.7
Rubisco activase	PMON82754	ZM_S110837	37.0	-3.4	3.9
Rubisco activase	PMON82754	ZM_S111476	37.0	-3.7	-0.2
Enhancerless rice actin	PMON82453	ZM_M87051	16.1	-4.2	0.7
rab17	PMON82454	ZM_S112701	15.7	-5.5	1.0
Enhancerless rice actin	PMON82452	ZM_M87438	14.8	-9.4	0.8
Enhancerless rice actin	PMON82452	ZM_M87010	14.3	0.6	-1.5
rab17	PMON82454	ZM_S112696	11.1	-6.3	0.5
rab17	PMON82454	ZM_S111896	10.9	-3.6	-2.0
Enhancerless rice actin	PMON82453	ZM_M88601	10.8	6.3	-4.1
Enhancerless rice actin	PMON82452	ZM_M87952	10.5	-2.5	-2.5

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
rab17	PMON82454	ZM_S112667	10.3	-7.0	-3.8
rab17	PMON82454	ZM_S110587	10.2	-3.2	-4.6
rab17	PMON82454	ZM_S110594	10.1	-4.3	0.4
Enhancerless rice actin	PMON82453	ZM_M87033	9.9	1.5	-1.0
rab17	PMON82454	ZM_S112682	9.7	0.7	-5.8
Enhancerless rice actin	PMON82453	ZM_M88129	9.5	0.4	0.0
Enhancerless rice actin	PMON82452	ZM_M87937	9.5	0.0	-6.9
rab17	PMON82454	ZM_S112657	9.2	-0.4	1.1
Enhancerless rice actin	PMON82452	ZM_M87441	9.2	2.0	-4.7
rab17	PMON82454	ZM_S112654	9.1	-8.3	0.9
Rice tubulin	pMON82752	ZM_M82769	9.0	-2.0	5.3
Enhancerless rice actin	PMON82452	ZM_M87000	8.9	-0.5	-8.3
Enhancerless rice actin	PMON82452	ZM_M87019	8.8	0.5	-0.8
Enhancerless rice actin	PMON82452	ZM_M85731	8.8	-1.8	-3.3
Enhancerless rice actin	PMON82453	ZM_M87036	8.7	-1.8	4.1
Enhancerless rice actin	PMON82453	ZM_M87027	8.4	2.2	0.3
Enhancerless rice actin	PMON82452	ZM_M85725	8.4	-9.6	1.1
rab17	PMON82454	ZM_S112713	8.3	10.2	0.1
Enhancerless rice actin	PMON82453	ZM_M87049	8.1	4.2	7.7
Enhancerless rice actin	PMON82453	ZM_M87378	8.0	0.8	5.8
Enhancerless rice actin	PMON82452	ZM_M87949	8.0	5.2	6.6
Enhancerless rice actin	PMON82452	ZM_M85734	8.0	-3.2	-4.8
Rice tubulin	pMON82753	ZM_M86065	7.8	-8.8	-8.9
Rice tubulin	pMON82752	ZM_M84408	7.6	-0.8	7.1

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
Enhancerless rice actin	PMON82453	ZM_M88602	7.6	5.9	-0.9
Rice tubulin	pMON82752	ZM_M82855	7.5	-35.2	3.4
Rice tubulin	pMON82752	ZM_M85712	7.5	-10.5	-6.5
Enhancerless rice actin	PMON82453	ZM_M88595	7.5	6.3	6.6
Rice tubulin	pMON82753	ZM_M83475	7.4	2.9	5.5
Enhancerless rice actin	PMON82453	ZM_M87335	7.4	0.4	9.6
Rice tubulin	pMON82753	ZM_M84105	7.2	-15.1	0.5
Rice tubulin	pMON82753	ZM_M84738	7.0	2.1	1.8
Rice tubulin	pMON82753	ZM_M84086	7.0	1.4	-2.2
rab17	PMON82454	ZM_S112714	7.0	0.4	-12.0
Enhancerless rice actin	PMON82452	ZM_M87427	7.0	1.1	-7.6
Rice tubulin	pMON82753	ZM_M84741	6.9	-11.3	-7.9
Enhancerless rice actin	PMON82453	ZM_M87052	6.9	-4.0	-7.6
Enhancerless rice actin	PMON82452	ZM_M87936	6.8	-6.7	-8.4
Rice tubulin	pMON82753	ZM_M86087	6.7	-11.0	-5.8
Rice tubulin	pMON82752	ZM_M83321	6.5	3.2	-5.7
Rice tubulin	pMON82753	ZM_M83478	6.4	-5.3	-8.0
Rice tubulin	pMON82752	ZM_M82773	6.4	0.4	10.5
Rice tubulin	pMON82752	ZM_M83306	6.4	-1.2	1.0
Rice tubulin	pMON82752	ZM_M84389	6.2	-1.5	3.9
Rice tubulin	pMON82752	ZM_M84393	6.1	0.9	-5.2
p326	PMON78305	ZM_S121096	6.1	2.6	-7.8
Rice tubulin	pMON82753	ZM_M86067	5.9	4.5	16.0
Rice tubulin	pMON82753	ZM_M83476	5.9	-6.7	11.8
Rubisco activase	PMON82754	ZM_S110873	5.8	-0.4	-6.0
Rice tubulin	pMON82753	ZM_M83470	5.8	-2.3	1.1
p326	PMON78305	ZM_S121092	5.8	-0.1	-5.4
NAS	PMON73610	ZM_S115519	5.8	4.0	8.1
Rice tubulin	pMON82752	ZM_M83933	5.7	-8.8	14.4
p326	PMON78305	ZM_S121064	5.7	3.5	-11.0
Rice tubulin	pMON82753	ZM_M84797	5.6	-3.6	11.7

Promoter	Vector	Event	pg NF-YB2 / µg total protein	Sufficient Water Yield Delta	Water Deficit Stress Yield Delta
Rice tubulin	pMON82752	ZM_M82772	5.5	-0.9	-6.0
p326	PMON78305	ZM_S121124	5.5	2.7	2.0
p326	PMON78305	ZM_S121122	5.4	0.9	4.2
p326	PMON78305	ZM_S121123	5.4	4.4	-11.7
p326	PMON78305	ZM_S121068	5.3	-0.7	4.0
NAS	PMON73610	ZM_S114691	5.3	6.5	5.5
Rice tubulin	pMON82753	ZM_M83465	5.1	0.4	-4.5
p326	PMON78305	ZM_S121130	5.0	-0.1	3.8
Rice tubulin	pMON82752	ZM_M82770	4.7	-14.3	5.6
NAS	PMON73610	ZM_S115769	4.6	8.9	5.2
NAS	PMON73610	ZM_S114480	4.4	4.2	4.2
NAS	PMON73610	ZM_S117076	4.4	1.8	2.6
NAS	PMON73610	ZM_S115703	4.4	-2.1	-0.7
NAS	PMON73610	ZM_S115567	4.0	-2.3	4.1
p326	PMON78305	ZM_S121070	3.9	-2.8	-5.8
NAS	PMON73610	ZM_S115721	3.9	1.7	7.2
p326	PMON78305	ZM_S121091	3.6	5.0	-3.6

Example 4

[0063] Transgenic cotton plants prepared as described in Example 1 comprising DNA constructs stably inserted in the chromosome and expressing an NF-YB protein under the control of promoters shown in Table 8 are evaluated for yield under water deficit stress and sufficient conditions.

Table 8 Promoter::NF-YB Constructs in Transgenic Cotton Plants

Promoter	Promoter SEQ ID	Protein	Vector
Enhanced CaMV 35S	SEQ ID NO:2	Arabidopsis G481	pMON83103
rd29a	SEQ ID NO:19	Arabidopsis G481	pMON95538
Tsf1	SEQ ID NO:60	Arabidopsis G481	pMON95559

[0064] Events of transgenic cotton plants comprising the above constructs and expressing the Arabidopsis NF-YB protein are grown under water deficit stress and sufficient water conditions and events are identified that have low leaf protein levels which impart improved yield (lbs/acre) as compared to wild type control plants when grown under water deficit stress conditions and comparable or improved yield as compared to wild type control plants when grown under sufficient water conditions.

Example 5

[0065] Transgenic soybean plants prepared as described in Example 1 comprising DNA constructs stably inserted in the chromosome and expressing an NF-YB protein under the control of promoters shown in Table 9 are evaluated for yield under water deficit stress and sufficient conditions.

Table 9 Promoter::NF-YB Constructs in Transgenic Soybean Plants

Promoter	Promoter SEQ ID	Protein	Vector
Soybean phaseolin	SEQ ID NO:61	Arabidopsis G481	pMON106646
Enhanced CaMV 35S	SEQ ID NO:2	Soybean G481-6	pMON83057
Enhanced CaMV 35S	SEQ ID NO:2	Arabidopsis G481	pMON63796

[0066] Events of transgenic soybean plants comprising the above constructs and expressing the Arabidopsis or soybean G481 NF-YB proteins are grown under water deficit stress and sufficient water conditions and events are identified that have low leaf protein levels which impart improved yield yield (bu/acre) as compared to wild type control plants when grown under water deficit stress conditions and comparable or improved yield as compared to wild type control plants when grown under sufficient water conditions.

Example 6

[0067] Transgenic alfalfa, canola, switchgrass, sugarcane and rice plants prepared as described in Example 1 comprising DNA constructs stably inserted in the chromosome and expressing an NF-YB protein under the control of promoters shown in Table 1 are evaluated for yield under water deficit stress and sufficient water conditions. Events of these transgenic plants are grown under water deficit stress and sufficient water conditions and events are identified that have low leaf protein levels which impart improved yield as compared to wild type control plants when grown under water deficit stress conditions and comparable or improved yield as compared to wild type control plants when grown under sufficient water conditions.

[0068] All of the materials and methods disclosed and claimed herein can be made and used without undue experimentation as instructed by the above disclosure. Although the materials and methods of this invention have been described in terms of preferred embodiments and illustrative examples, it will be apparent to those of skill in the art that variations may be applied to the materials and methods described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

What is claimed is:

1. A plant chromosomal DNA segment comprising a recombinant polynucleotide flanked by native plant DNA, wherein said polynucleotide provides for expression of at least an NF-YB protein and a marker protein, and wherein said NF-YB protein is produced in leaf cells of said plant at a level up to 40 picograms per microgram of total protein in said plant leaf tissue cells.

2. A plant chromosomal DNA segment comprising a recombinant polynucleotide flanked by native plant DNA, wherein said polynucleotide provides for expression of a single protein, wherein said protein is an NF-YB protein, and wherein said NF-YB protein is produced in leaf cells of said plant at a level up to 40 picograms per microgram of total protein in said plant leaf tissue cells.

3. A plant chromosomal DNA segment comprising a recombinant DNA construct for expressing an NF-YB protein comprising contiguous amino acids from SEQ ID NO:28, wherein said amino acids include:

(a) amino acids at position 49 thorough 122 of SEQ ID NO:28 and wherein one or more amino acids at position number 49, 73, 76, 83, 89, 102, 103, 109, 115, 118 or 122 are different; or

(b) amino acids at position 49 thorough 122 of SEQ ID NO: 28 and wherein one or more amino acids at position number 49, 73, 76, 83, 89, 102, 103, 109, 115, 118 or 122 are different and one or more of amino acids at position 55 through 61 are missing;

(c) amino acids of SEQ ID NO: 28 at position 29 through 134 and one or more of amino acids at position 2 through 28 are missing; or

(d) amino acids of SEQ ID NO: 28 at position 68 through 134 and one or more of amino acids at position 2 through 67 are missing.

4. A plant chromosomal DNA segment of any of claims 1-3 wherein said recombinant polynucleotide comprises a promoter is selected from the group consisting

of a rice alpha tubulin promoter, a rice actin promoter, a PPDK mesophyl tissue enhanced promoter, and a rubisco activase bundle sheath enhanced promoter.

5. A plant chromosomal DNA segment of any of claims 1-3 and wherein said NF-YB protein is produced in leaf cells of said plant at a level between 0.1 and 11 picograms per microgram of total protein in said plant leaf tissue cells.

6. A transgenic plant cell comprising a plant chromosomal DNA segment of any of claims 1-3.

7. A transgenic crop of water deficit stress tolerant plants comprising cells of claim 6 wherein the harvested yield of said crop is comparable to or enhanced over the yield of a crop of control plants not having said plant chromosomal DNA segment when said crops are grown in water sufficient conditions.

8. A transgenic crop of Claim 7 wherein said water deficit stress tolerant plants are corn, cotton, soybean, sugarcane, switchgrass, rice, wheat, alfalfa, or canola plants.

9. A transgenic corn plant seed comprising a plant chromosomal DNA segment of any of claims 1-3 wherein said NF-YB protein is a native corn protein.

10. A method of improving water stress tolerance and yield in a crop plant line comprising providing in the genome of said crop plant line a plant chromosomal DNA segment of any of claims 1-3.

11. A transgenic pollen grain comprising a haploid derivative of a plant cell containing a a plant chromosomal DNA segment of any of claims 1-3.

12. A method for manufacturing non-natural, transgenic seed that can be used to produce a crop of transgenic plants with enhanced water deficit stress tolerance resulting

from expression of an NF-YB protein from a plant chromosomal DNA segment of any of claims 1-3, wherein said method comprises:

(a) screening a population of plants having said plant chromosomal DNA segment and control plants for said enhanced yield when grown under deficit stress or enhanced or comparable yield as compared to the yield for control plants when grown under sufficient water conditions,

(b) selecting from said population one or more plants that exhibit enhanced yield as compared to the yield for control plants under water deficit stressed or enhanced or comparable yield as compared to the yield for control plants when grown under water sufficient conditions,

(c) verifying that said plant chromosomal DNA segment is stably integrated in said selected plants,

(d) analyzing leaf tissue of a selected plant to determine the production of transgenic NF-YB protein at a level up to 40 picograms of NF-YB protein per microgram of total protein in said leaf tissue; and

(e) collecting seed or a regenerative propagule from a selected plant.

13. A method of claim 12 wherein said seed is corn, cotton, soybean, sugarcane, switchgrass, rice, wheat, alfalfa, or canola seed.

14. A method of producing inbred corn seed comprising:

(a) acquiring hybrid corn seed from a herbicide tolerant corn plant which also has stably-integrated, chromosomal DNA segment of any of claims 1-3;

(b) introgressing the chromosomal DNA segment from said acquired hybrid corn seed into a second corn line by allowing pollen grains comprising a haploid derivative with said chromosomal DNA segment to pollinate said second corn line to produce crossed seeds ,

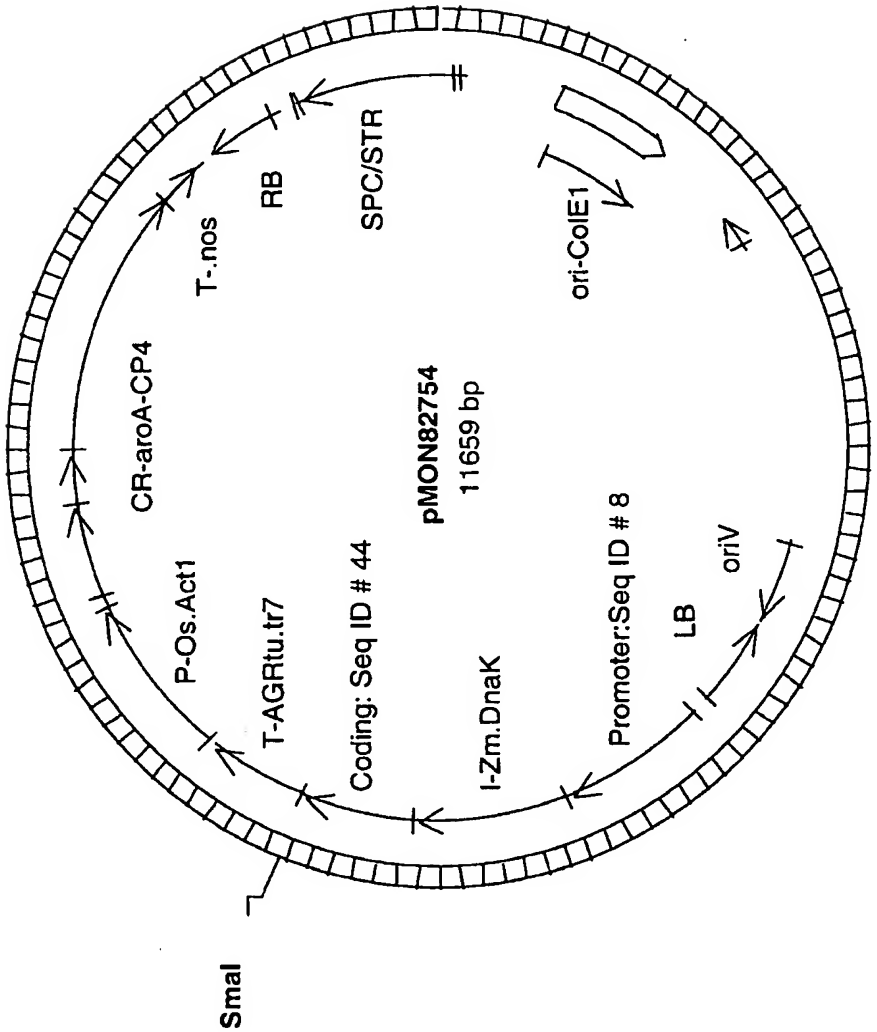
(c) producing a population of plants from crossed seeds wherein a fraction of the seeds produced from said pollination is homozygous for said chromosomal DNA segment, a fraction of the plants produced from said hybrid corn seed is hemizygous

for said chromosomal DNA segment, and a fraction of the plants produced from said hybrid corn seed does not have said chromosomal DNA segment;
(d) selecting corn plants which are homozygous and hemizygous for said chromosomal DNA segment by treating with an herbicide;
(e) collecting seed from herbicide-treated-surviving corn plants and planting said seed to produce further progeny corn plants;
(f) backcrossing plants grown from said progeny seeds with said second corn line to produce an inbred corn line.

15. The method of claim 14 further comprising crossing said inbred corn line with a third corn line to produce hybrid seed.

16. Anti-counterfeit milled seed having, as an indication of origin, a plant cell with said chromosomal DNA segment of any of claims 1-3.

17. A method of growing a corn, cotton, soybean, sugarcane, switchgrass, rice, wheat, alfalfa, or canola crop without irrigation water comprising planting seed having plant cells with a plant chromosomal DNA segment of any of claims 1-3 which are selected for enhanced water deficit tolerance.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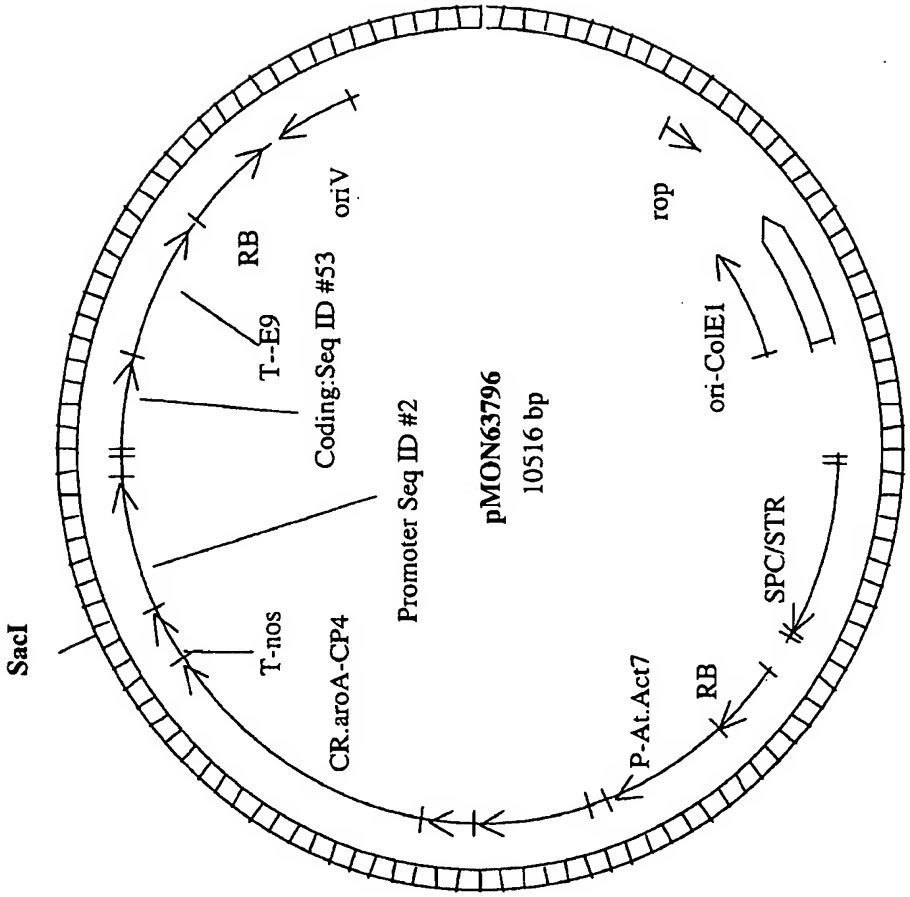
Promoter:Seq ID # 8
LB
oriV
ori-ColE1
SPC/STR
RB
T-.nos

FIGURE 1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RB
oriV
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ori-ColE1
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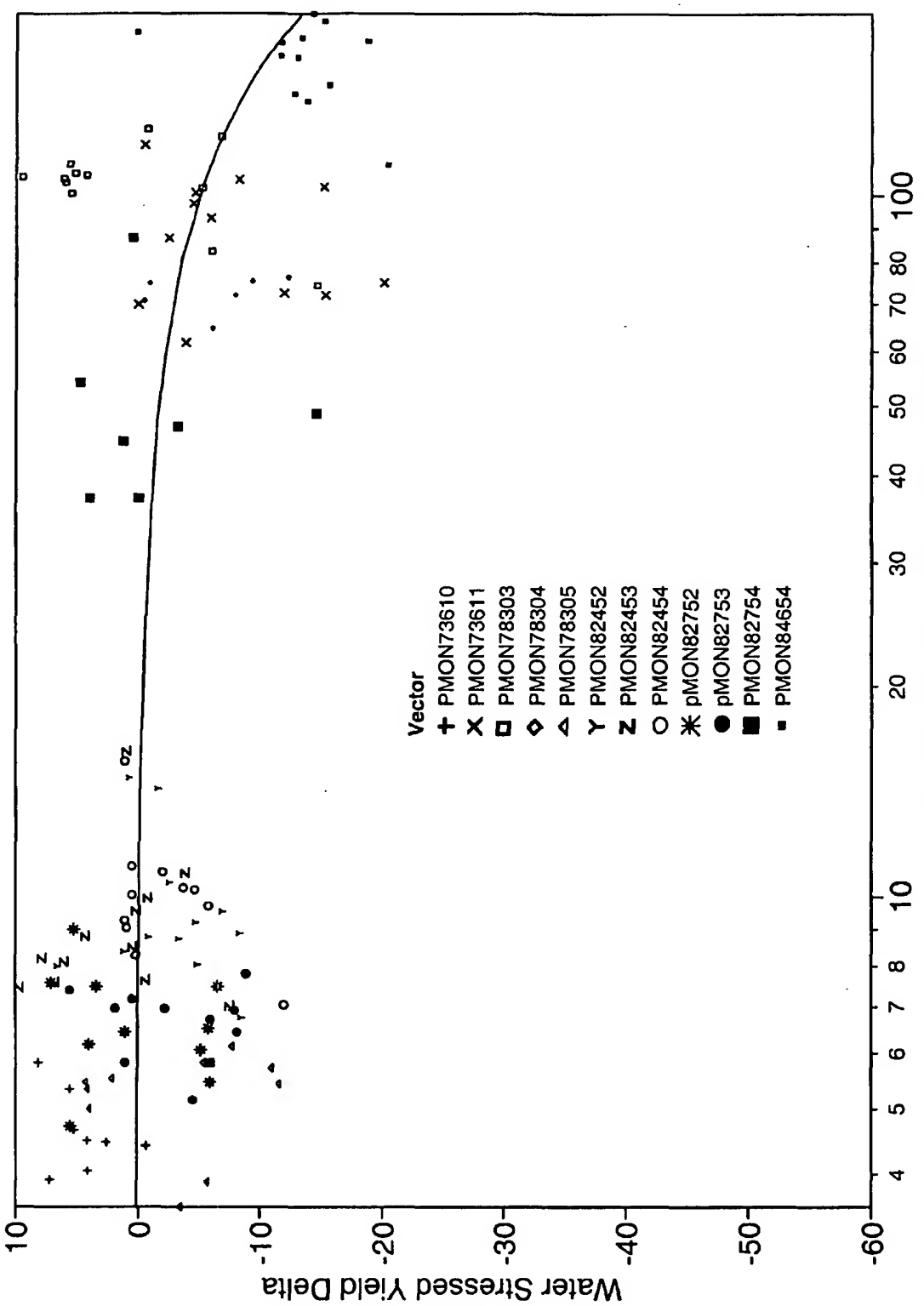
P-At.Act7

FIGURE 2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0
-10
-20
-30
-40
-50
-60
4
5
6
7
8
10
20
30
40
50
60
70
80
100
pg NFB2 per microgram total protein
Vector
PMON73610
PMON73611
PMON78303
PMON78304
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pMON82753
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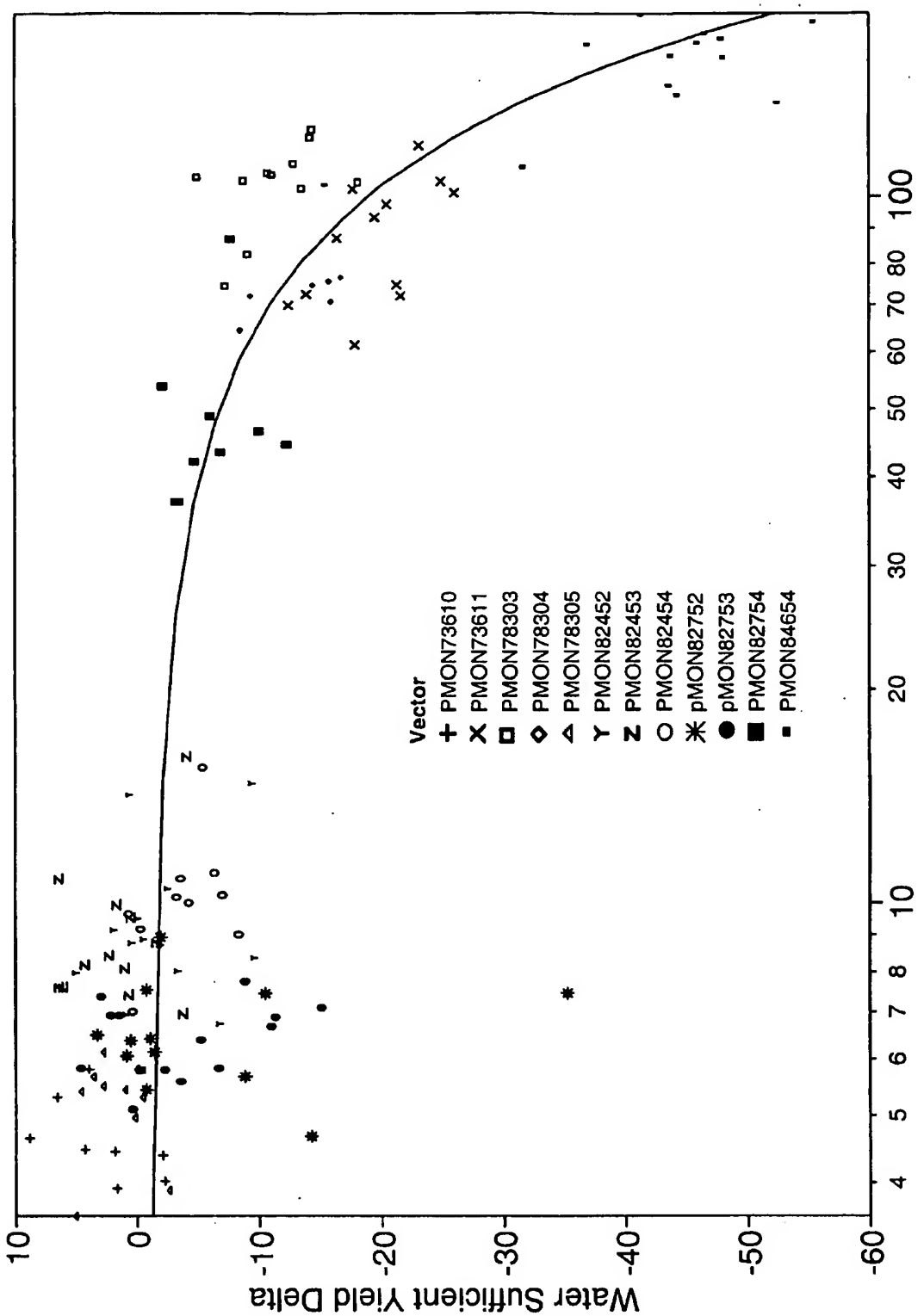
FIGURE 3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0
-10
-20
-30
-40
-50
-60
4
5
6
7
8
10
20
30
40
50
60
70
80
100
pg NFB2 per microgram total protein
Vector
PMON73610
PMON73611
PMON78303
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PMON82453
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pMON82752
pMON82753
PMON82754
PMON84654

FIGURE 4